# Ultrastructural Changes in Rat Liver Cells Following a Single Oral Dose of TCDD

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Ultrastructural alterations of liver parenchymal cells involving the endoplasmic reticulum are known to occur in animals exposed to chlorinated diphenyl-*p*-dioxins in the diet (*1–4*). The observed changes are similar to those reported for other aromatic chlorinated hydrocarbons (*5–8*) and are usually associated with induction of hepatic microsomal enzyme systems (*7, 9, 10*).

The present study was undertaken to correlate ultrastructural changes in rat liver cells with biochemical studies on liver microsomes and mitochondria for a period of 28 days following a single oral dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

## Materials and Methods

Ninety male Charles River rats were separated into three groups of 30 each. Animals in the first group received a single oral dose by gavage of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in 0.5 ml of acetone and corn oil at a dose of 5 μg/kg while the second group was given a dose of 25 μg/kg. The third group served as controls. Five animals from each group were killed by decapitation at 1, 3, 6, 9, 16, and 28 days after treatment.

Blocks of liver tissue were fixed in buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light microscopy. Other blocks of liver were placed in a glutaraldehyde-formaldehyde fixative described previously (*11*), embedded in Epon, and sectioned with diamond knives. These sections were double-stained with lead citrate and uranyl acetate prior to examination in a Philips EM 300.

## Results

Histologic sections of liver from all treated animals were indistinguishable from controls. Liver parenchymal cells of all animals killed on the first day after treatment exhibited ultrastructural architecture similar to controls (Fig. 1). By the third day, liver cells of treated animals contained increased amounts of smooth endoplasmic reticulum (SER) which were prominent around the periphery of cells particularly in areas adjacent to bile canaliculi (Fig. 2). More SER was observed in rats given the higher dose of TCDD. Some cells of treated animals also appeared to contain more rough endoplasmic reticulum (RER) than controls.

Large aggregates of SER and massive amounts of RER (Fig. 3) were observed in the liver cells of treated animals killed on days 6 and 9. This observation was most readily appreciated in rats given the

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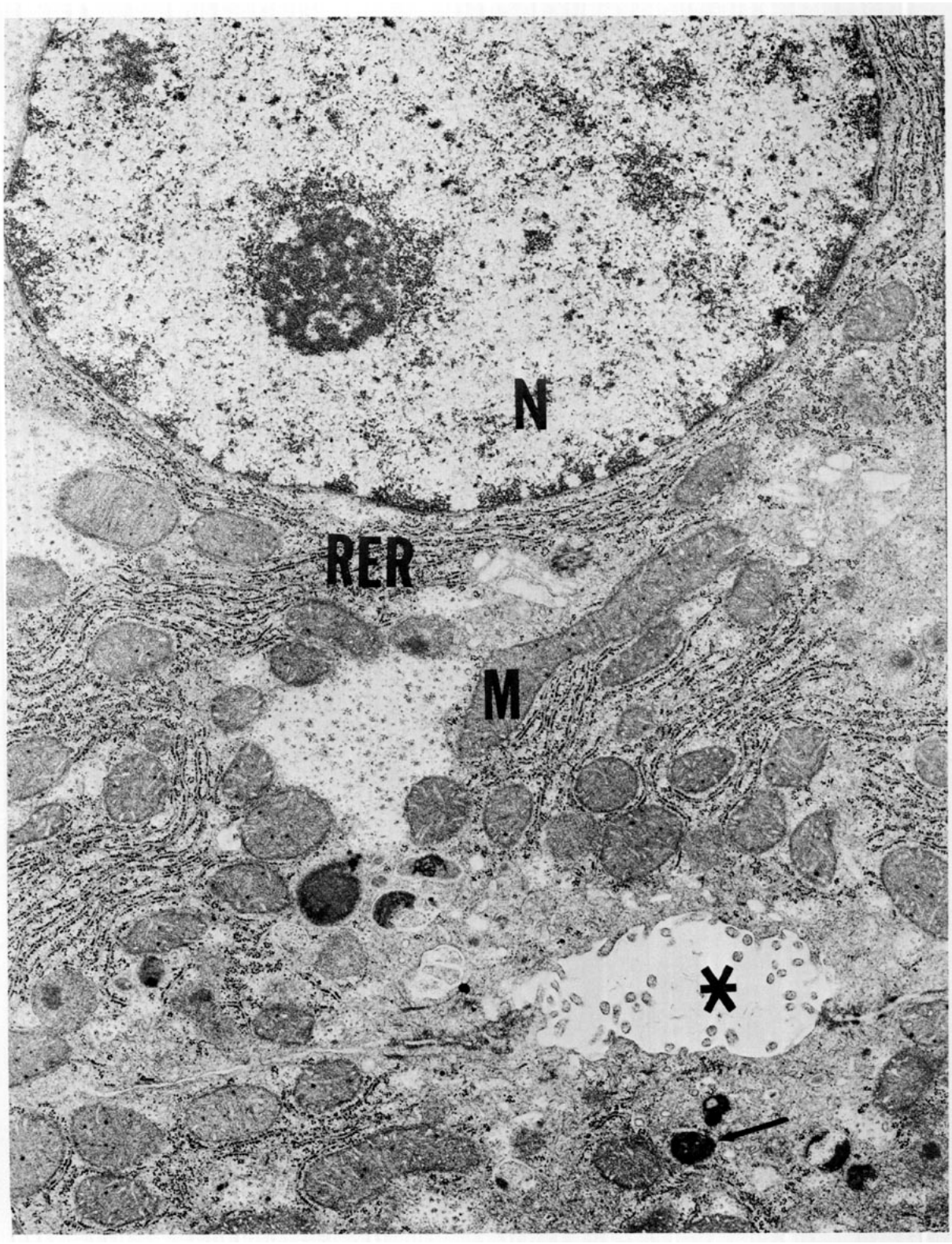


FIGURE 1. Liver parenchymal cell from an animal killed 1 day after dioxin treatment displaying usual architecture. Nucleus (N) mitochondria (M), rough endoplasmic reticulum (RER), cytosomes (arrow) and bile canaliculus (*) are present in the cytoplasm. 15,102×.

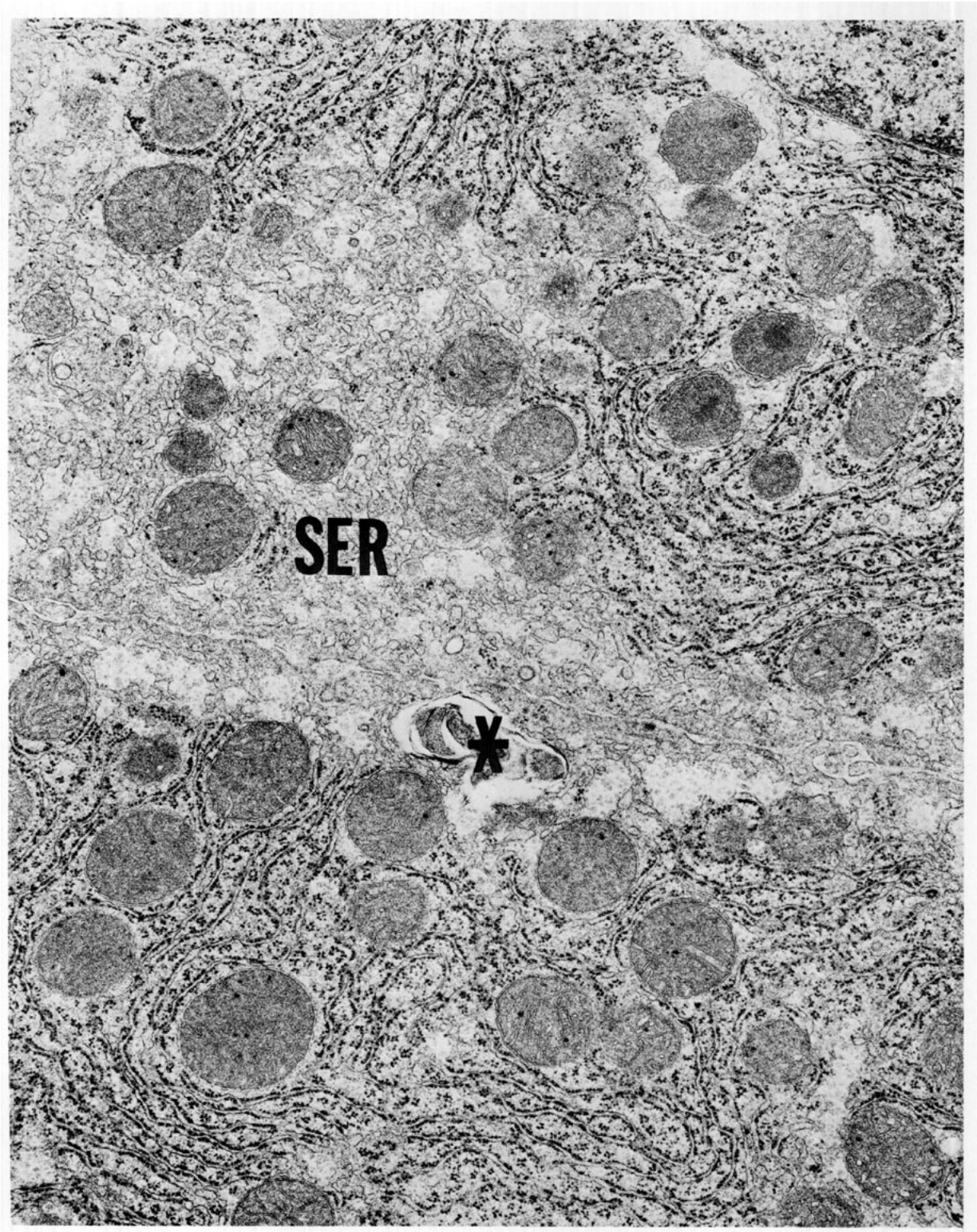


FIGURE 2. Liver cell from a rat 3 days after exposure to dioxin. Increased amounts of smooth endoplasmic reticulum (SER) are present around a bile canaliculus (*). The RER also seems to be more prominent. 20,520×.

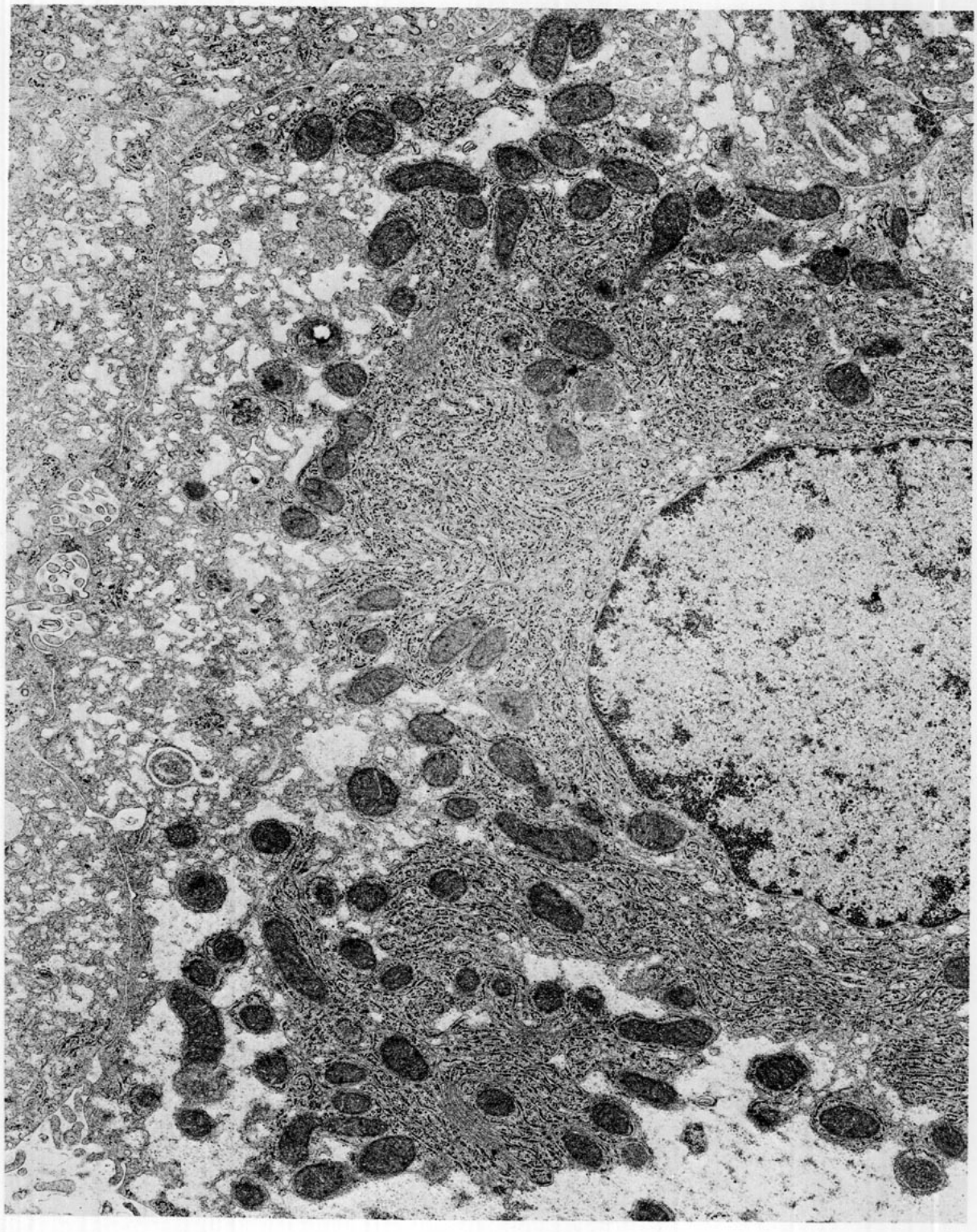

FIGURE 3. Cell from a rat killed 6 days after administration of dioxin. Large amounts of SER are seen around the cellular periphery and massive amounts of RER surround the nucleus. 11,080×.

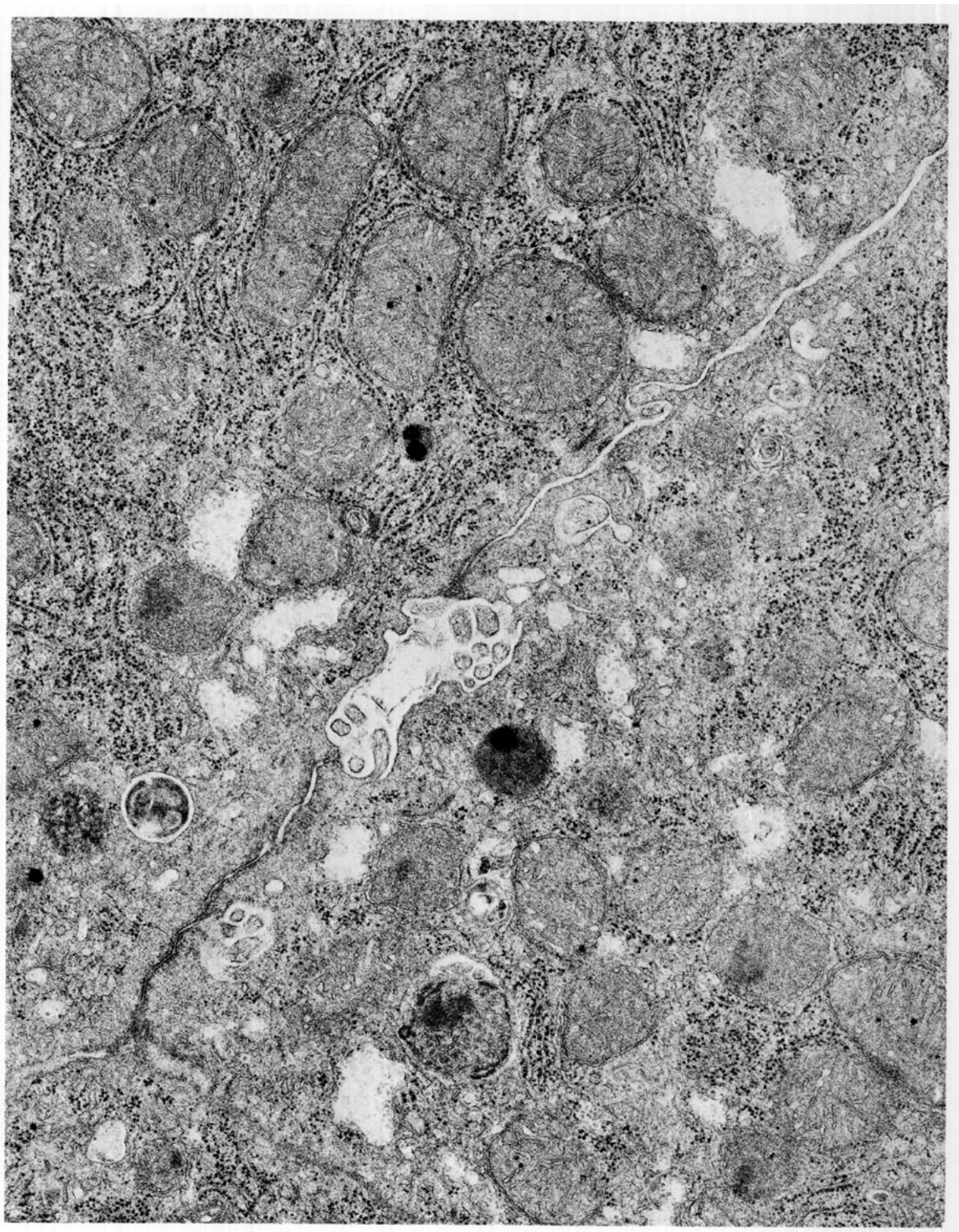

FIGURE 4. Parenchymal cell from a rat 16 days after treatment. Large amounts of RER are still prominent in the cytoplasm but the SER is greatly diminished. 20,520×.

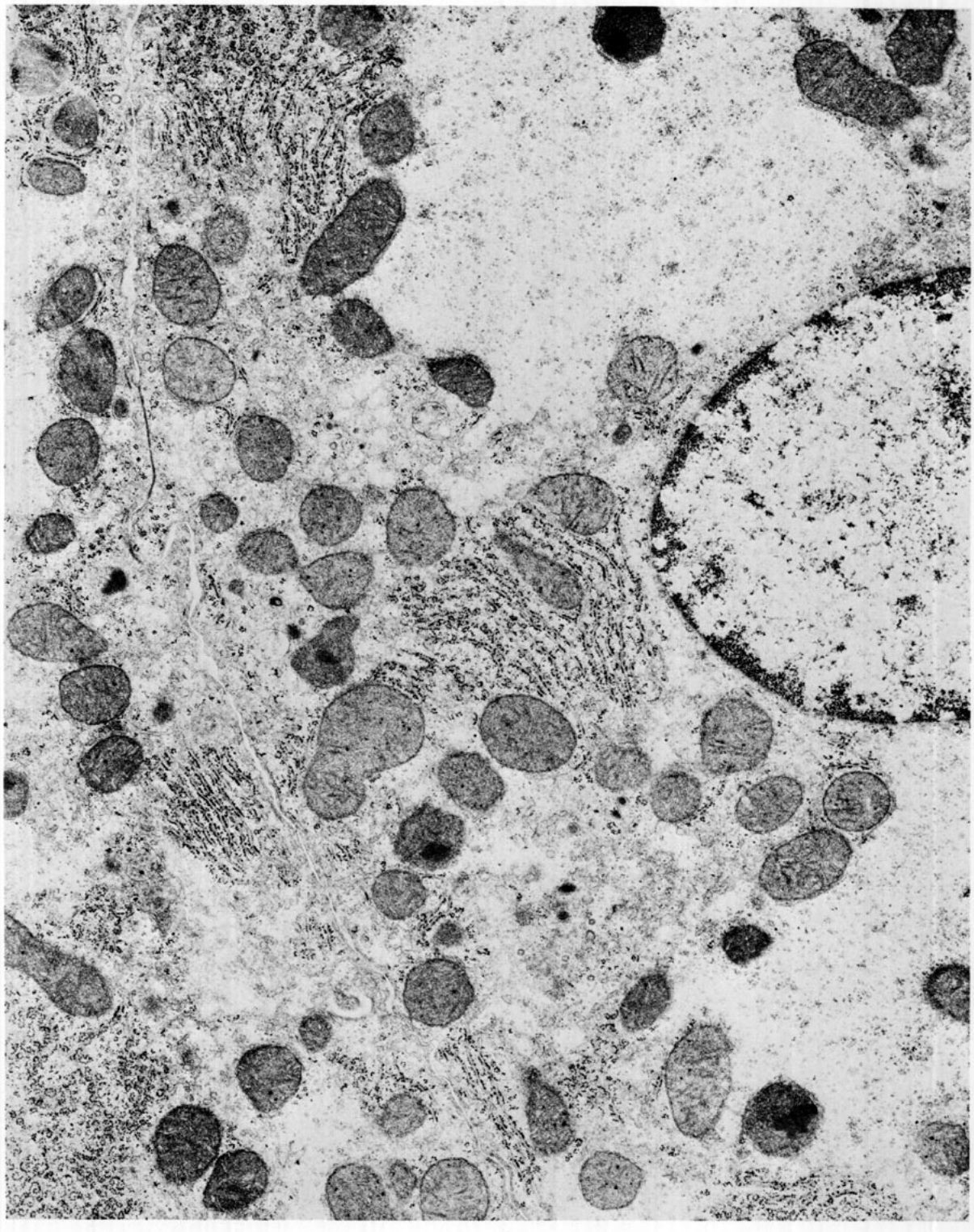

FIGURE 5. Liver cells of a rat killed 28 days after treatment containing levels of endoplasmic reticulum comparable to controls. 1510×.

higher dose of TCDD. Parenchymal cells of rats killed on day 16 contained little SER but large amounts of RER were still evident in many cells (Fig. 4). On the day 28, most liver cells of treated animals were indistinguishable from controls (Figure 5). Membranous concentric whorls of endoplasmic reticulum described in other studies (*4*) were never observed in liver cells of any treated animals during the course of the experiment.

## Discussion

Proliferation of SER in liver cells of rats given TCDD confirms previous reports by other investigators (*4*). The increase of SER in parenchymal cells of treated animals used in this study was closely correlated with induction of microsomal enzyme activity in livers of these same animals (*12*). Similar observations have been made for a wide variety of other aromatic chlorinated compounds (*7, 9, 10*) and are indicative of a cellular attempt at detoxification.

Early proliferation of SER around bile canaliculi of liver cells from treated animals could suggest involvement of bile in the metabolism or excretion of TCDD. This idea is supported by the fact that increased bile secretion was noted (*13*) in other animals used in this study and rats given labeled octachloro dioxin excreted most of the radioactivity in the feces (*14*).

Alterations in RER associated with the formation of membranous concentric whorls of endoplasmic reticulum (*3, 4*) have been noted in livers of animals exposed to chlorinated diphenyl-*p*-dioxins in the diet. In the present study, proliferation of RER reached a maximum at day 6 following administration of TCDD and was correlated with increased RER as determined by microsomal subfractionation (*12*). These observations would suggest a concomitant stimulation of both RNA and protein synthesis within liver cells of treated animals. The significance and mechanism of this are unclear, but they might be related to increased synthesis of microsomal proteins (*3*).

The lack of membranous concentric whorl formation in liver cells of TCDD-treated rats used in this study may suggest that chronic exposure and accumulation of TCDD or its metabolites within liver cells is necessary for the phenomenon to occur. Another possibility is that other dioxins or aromatic chlorinated compounds rather than TCDD were responsible for the membranous whorls observed in earlier studies. Investigation utilizing different doses, other purified dioxin compounds, and dioxin tissue analyses may be necessary to resolve this question.

In conclusion, a single low-level dose of TCDD has been found to exert a profound effect on both the SER and RER of rat liver parenchymal cells. At present, the nature of these changes appears to be related to an induction phenomenon and changes in cellular RNA and protein metabolism. Many other studies are needed to examine the mechanisms responsible for these alterations in normal cellular function.

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